Factors associated with antiretroviral resistance in human immunodeficiency virus patients on antiretroviral therapy in South Africa

Dickman Pangaume Gareta

A research report submitted to, the Faculty of Health Sciences, University of Witwatersrand, in partial fulfilment of the requirements for the degree

of

Master of Science

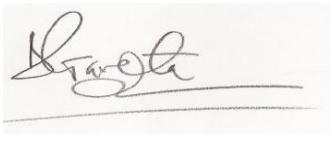
in

Population based field epidemiology

March, 2013

Declaration

I, ***Dickman Pangaume Gareta***, declare that this is my own work. It is being submitted for the degree of Master of Science in Epidemiology in the field of Population Based Field Epidemiology in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature: 

Full Name: Dickman Pangaume Gareta.

Date: 26th Day of February, 2013

Dedication

I dedicate my MSc degree to my mom, Rose Gareta who has persevered through thick and thin so that I should be where I am now. I would like to say *muchas gracias, me has ayudado mucho, te doy gracias por el regalo.* I also dedicate this work to my late father Victor Gareta who was my source of inspiration throughout my life.

Again, I would like to dedicate this work to my lovely girlfriend Chifundo Santhe who gave me unconditional love and support during my studies; my one and only brother Paul for his words of encouragement; my late brothers Steven and Beston for instilling sense of confidence in me.

Most importantly, I thank the Lord God Almighty for giving me wisdom and knowledge and making it possible that my MSc degree should be a success. Glory and honour be unto Him.

Abstract

Introduction: Access to highly active antiretroviral therapy has dramatically increased worldwide since 2004. However, the emergence of HIV drug resistance presents huge obstacle in ART scale up as it contributes to treatment failure and poses a greater risk of disease progression and loss of treatment options. The study therefore investigated the risk factors and the association of HIV drug resistance, virological failure and CD4 cell count changes in patients on ART at Aurum Institute for Health Research in South Africa.

Methods: A cohort of HIV infected patients who developed virological failure of their first HAART regimen was assessed. A genotypic resistance testing was performed using stored plasma on a subset of patients at first detection of virological failure. Data were collected prospectively on all registered patients using standardised forms. Clinical data was obtained from laboratory and pharmacy electronic records. Logistic regression and Cox proportional hazard models were used to assess factors associated with HIV drug resistance and virological failure respectively. Linear mixed-effects regression models were used to assess the changes in the CD4 cell count among patients who developed HIVDR.

Results: Between January 2003 and December 2010, a total of 146 ART-treated patients who experienced virological failure were assessed. Of these, 108 (74%) developed HIVDR, of whom 80 (74%) were males; the median CD4 cell count at ART initiation was 121 cells/mm^3 (interquartile range, 61-210). The most frequent NNRTI mutations patterns found were mutations leading to resistance to NNRTI agents with 33% having NNRTI resistance. The second most common resistance

pattern was resistance to lamivudine conferred by the M184V mutation (30%). The multivariable analysis showed that higher CD4 cell count at HIVDR detection was significantly associated with the reduced odds of developing HIV drug resistant mutation after adjusting for gender and age(adjusted OR=0.37, 95% CI 0.15–0.94). Similarly, there was significant association between age at ART initiation (adjusted HR=0.71, 95% CI 0.52–0.97) and CD4 cell count during follow-up (adjusted HR= 0.54 95% CI 0.36–0.81) with virological failure in those patients who developed HIVDR. The CD4 cell count slope on average increased by 10 cells per mL per year for the patients without any resistance (average annual change 9.89 cells per mL, 95% CI -6.90-26.69) and decreased by 10 cells per mL per year for patients who had any resistance (average annual change -9.61 cells per mL, 95% CI -19.41- 0.17).

Conclusion and recommendation: HIV drug resistant virus was found in 74% of the South African patients who were accessing HIV care at Aurum Heath Institute and developed virological failure of first HAART regimen. Higher CD4 count at detection of HIVDR was significantly associated with lower risk of developing HIV drug resistant virus. Lower CD4 count and male gender were significantly associated with the development of virological failure. Patients with virological failure had significantly great CD4 count declines when any mutation and thymidine analog mutation (TAM) mutation were present. There is a great need therefore for multifaceted approach to target interventions that aim to increase patients CD4 cell counts. Patients should be either screened, possibly with HIVDR testing, prior to re-initiation of a first-line regimen.

Keywords: HIV; drug resistant virus; antiretroviral; virological failure; CD4 slope.

Acknowledgements

I am greatly indebted to my sponsor Malawi National Commission for Science and Technology (NCST) for the award of Health Research Capacity Strengthening Initiative (HRCSI) scholarship without which I would never have been able to pursue my MSc degree course.

I would like to express my deepest gratitude to my supervisors Dr. Edmore Marinda of the School of Public Health, University of Witwatersrand and Dr. Christopher J. Hoffmann of Aurum Institute for Health Research (Aurum) for their excellent supervision, guidance, patience and support.

I would also like to extend my sincere thanks to the Chief Executive Officer and members of staff of Aurum for providing the dataset for this research as well their support. Special thanks to Kavindhran Velen for the support he rendered throughout the research.

I want to seize this opportunity to thank all lecturers in the School of Public Health, University of the Witwatersrand, for their academic support during my study. Specifically, I would like thank Dr. Tobias F.Chirwa for introducing me to the world of statistic.

Finally, I would to thank my 2011 classmates especially Alfred Kwesi Manyeh for the fun, encouragement and support. Dr. B. Ikalafeng and Georgina Badu-Gyan for the encouragement rendered during the writing of the report.

Table of Contents

List of Tables

List of Appendices

List of Abbreviations and Acronyms

AZT	Zidovudine
D4T	Stavudine
EFV	Efavirenz
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency virus
HIVDR	HIV drug resistance
MDR	Multidrug resistant tuberculosis
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors
NVP	Nevirapine
PI	Protease inhibitors
RNA	Ribonucleic acid
TAM	Thymidine analog mutation
TB	Tuberculosis
TDF	Tenofovir
VL	Viral load
WHO	World Health Organisation
XDR	Extensively drug resistant tuberculosis
3Tc	Lamivudine

Chapter 1 : Introduction

1.1. Background

Access to highly active antiretroviral therapy (HAART) has dramatically increased in low and middle-income countries since 2004. By the end of 2009, over 5 million people were receiving antiretroviral therapy (ART) in these countries, a 13-fold increase since 2004(1). In Sub Saharan Africa, the scale up of ART treatment has profoundly benefited majority of people living with human immunodeficiency virus (HIV)(1). In 2009, 37% of adults and children eligible for ART were receiving it in the region compared to only 2% seven years earlier(2). This expansion in ART access has in turn contributed to a 19% decline in deaths among people infected with HIV between the period 2004 and 2009(1).

The long term success of ART may however be impeded by the emergence of HIV drug resistance (HIVDR). The development of HIVDR among HIV infected patients remains an important challenge in the management of HIV disease since this contributes to treatment failure(3) and poses high risk of disease progression and results in limited treatment options. HIVDR can lead to increased health costs associated with the need to start more costly second-line treatment for patients, the spread of resistant strains of HIV and the need to develop new anti-HIV drugs(4). Therefore emergence of HIVDR is a major concern as it may likely lead to a situation in which no effective drugs are available for the treatment of Acquired Immune Deficiency Syndrome (AIDS).

In South Africa, the Comprehensive Plan for the Care, Management and Treatment of HIV and AIDS, was initiated in 2003(5). The objective was to achieve universal ART access(5). By the end of 2009, more than 900 000 of the approximately 6 million HIV infected individuals, were enrolled into ART program(6). The most common first-line HAART regimen used in South African ART programmes is the combination therapy, which comprises of two nucleoside reverse transcriptase inhibitors (NRTI) and one non-NRTI (NNRTI)(7). This is in accordance with World Health Organisation (WHO) recommendations which are based on efficacy, simplicity, tolerability, ease of administration and standardisation(8, 9). Although these regimens are highly effective, there are reports already of treatment failure in South African HIV patients(10). Therefore as the roll out of ART continues in developing countries, drug resistance monitoring of HIV strains will become increasingly important.

1.2. Literature Review

An increasing number of HIV infected individuals are benefiting from ART scale-up programmes. However the emergence of HIV resistant variants threatens this benefit. HIVDR substantially reduces treatment options. In addition, continuation of a regimen to which drug resistance exists is unlikely to lead to limited suppression levels leading to accumulation of HIVDR mutations. Such mutations may compromise subsequent ART regimens. While studies on HIVDR are well documented, the relationship between HIVDR and changes in CD4 count and/or viral load is still complex and not completely understood among HIV infected individuals. The objective of this chapter is to review relevant literature with the aim of identifying

and critiquing the most important issues involved in HIVDR among HIV-infected individuals.

1.2.1. Transmission of HIV drug resistance

The rate of transmitted drug resistant HIV is reported to be limited in low and middle income countries despite widespread use of ART. According to the WHO combined surveys conducted between 2003 and 2009 in twenty countries in Africa, Asia and Mexico, the overall rate of transmitted drug resistant HIV was about 4%(11). In Botswana, using a model of the stochastic evolution of drug-resistant HIV strains it was observed that transmission of drug-resistant strains would reach 15% by 2009 if evolving drug-resistant strains were as fit as the wild-type strains. This was however still low compared to the rate of transmission shown in developed countries where prevalence of transmission of drug resistant HIV was as high as 18%(12). One possible explanation for the high transmission prevalence in developed countries suggested by the authors was the widespread availability of ART drugs for a long period of time in these countries. With the increasingly widespread use of ART in developing countries, the trend observed in developed countries is likely to also be seen in these countries.

Previous studies have shown that acquired HIV drug resistant viruses are higher in developed countries compared to developing countries. Surveys of acquired HIV drug resistance conducted at clinics in Burundi, India, Malawi, Mozambique and Nigeria between the period of 2007 and 2010 showed that 6% of 2,150 patients initiating first line ART drugs at these clinics had one or more baseline drug resistance mutations(11). The observed HIV drug resistance prevalence rate was

low in these countries compared to the rates observed in the developed countries and could be attributed to limited availability of assays for routine determination of plasma HIV-1 RNA levels and for detecting drug resistance in these countries.

1.2.2. Prevalence and patterns of HIV drug resistance

While there are efforts by WHO and UNAIDS to monitor HIVDR and provide guidelines for preventing HIVDR in both the developed and developing countries, HIVDR remains considerably high. In the USA and France, genotypic data performed in HIV infected adults showed that 80% of the patients had virus strains resistant to at least one ART drug(13, 14). Similarly in resource poor settings, studies have shown levels of HIVDR as high as 80% in those failing ART (15-19). However, Harrigan *et al* suggested that prevalence of HIVDR should always be interpreted with caution as they are usually confounded by many factors(20). The author further suggested that this is because the denominator of all treated patients is often not known and the practice of obtaining drug resistance test results changes over time. Usually cross-sectional analyses are used to analyse prevalence of HIVDR and this may underestimate the prevalence of HIVDR(20).

Studies from Sub-Saharan Africa have reported high frequencies of drug resistant mutations in patients failing first line ART. Hosseinipour *et al* observed that 93% of the patients with failure of first-line ART comprising Stavudine, Lamivudine and Efavirenz in Malawi had NNRTI mutations and 81% had M184V mutations(15). In the same study, 17% of the patients demonstrated resistance to all NRTI currently available drugs and 56% had additional Thymidine analog mutation (TAM). Similarly, Shepherd *et al* also reported that 53% of patients on a failing ART regimen in

Nigeria had no active NRTI available to them(21). Related to this, Wallis *et al* found out that the most common reverse transcriptase mutation was M184V/I (72%)(10).This was in line with an observation noted by Hosseinipour *et al* and Marconi *et al* in which they found that the most common and frequent pattern of mutations found in developing countries were M184/I, NNRTI and TAM(15, 16). Y181C was found to be the most common mutation contributing to the resistance of NNRTIs(22). The high prevalence of HIVDR in these countries could be due to the widespread availability of ART drugs for substantial periods of time (15).

1.2.3. Risk factors associated with HIV drug resistance

Previous studies which have assessed the risk factors associated with emergence of HIVDR have shown differing results. Some studies have shown that HIVDR was associated with previous use of ART therapy, haemoglobin level, advanced HIV disease, history of injection drug use, CD4 cell count, high plasma HIV viral load(14, 23, 24). However, other studies have shown that age, gender, previous ART use, WHO stage, haemoglobin level, employment status, ethnicity, AIDS diagnosis, calendar year of initiation of HAART, type of HAART at initiation (NNRTI vs. Protease inhibitors(PI) based) were not associated with HIVDR(16, 24).

CD4 count and plasma viral load appear to be important predictors of HIVDR among patients accessing ART. It has been shown that patients with low CD4 count are associated with the development of HIV resistant strains(14, 15, 24).However, in another study of a cohort of 239 persons diagnosed with HIV, the results showed that higher CD4 count was associated with the emergence of HIVDR virus(25).

Richman *et al and* Harrigan *et al observed* that patients with higher plasma viral load were at a higher risk of developing HIVDR virus (14, 24). However, such finding was not in consistency with studies conducted in both ART naive and ART experienced patients (16, 26).

Adherence to ART treatment, previous ART use and advanced HIV disease are also associated with the development of HIVDR (14, 24, 26). Patients with ART adherence of less than 90 % were likely to develop HIVDR virus (24). In the multicentre Italian Antiretroviral Resistance Cohort Analysis (ARCA) study previous ART use was significantly associated with emergence of drug resistance virus (27).

HIVDR is a well-recognised obstacle in the ART scale up especially in South Africa where it has the largest public ART programme. Hence this research project aimed to describe risk factors associated with HIVDR and to assess the association between CD4 cell count slope and resistant HIV mutations.

1.3. Aims and objectives of the study

The principal aim of the study was to assess factors associated with the emergence of HIV drug resistant viruses among patients accessing ART at Aurum Institute for Health Research HIV workplace programme in Johannesburg South Africa between January 2003 and December 2010. The research report also aimed to assess the association of HIV drug resistance mutations and virological failure on the CD4 slope during persistent viremia.

1.3.1. Specific objectives

1. To describe baseline characteristics of clinical, immunological and virological failure patients starting first line HAART regimens in Johannesburg, South Africa between 2003 and 2010.
2. To describe HIVDR patterns among individuals with testing results either obtained for clinical use or for prior research studies.
3. To assess factors associated with the emergence of HIVDR virus strains.
4. To assess factors associated with virological failure in patients with HIVDR virus.
5. To investigate the relationship between changes in CD4 cell count with
    a. HIVDR among patients with HIVDR test results
    b. HIV RNA failure thresholds (>1000 copies / mL).

1.4. Justification of the study

As the roll out of ART continues in developing countries, drug resistance will predictably continue to emerge. It is suggested that even more extensive drug resistant viruses may emerge in these settings more than in the developed countries (28). As a result, costs involved in treatment and management of patients with drug resistant HIV strains as well as the costs associated with the development of new drugs will rise. In addition, with the rapid replicating mutants, new mutants which may be difficult to treat may evolve. Therefore, monitoring the development of HIV drug resistant strains will become increasingly important, and finding strategies for prevention of HIV resistance will be equally crucial. Early detection of drug resistant viruses will provide more treatment options. It would allow closer follow-up and more targeted interventions in high risk patients, thus improving patient treatment outcomes. Furthermore, the results from this study may offer additional findings to develop a strong base for policy framework and implementation of comprehensive HIVDR activities in the South African ART programme.

1.5. Outline of the research report

This report is organized into five chapters. The second chapter is methodology which provides a brief description of the study settings, study design, study population, data collection and statistical analysis. Chapter three presents the study results. Chapter four is discussion of the study results. Finally, chapter five is the conclusion of the results as well as remarks and policy recommendations based on the study findings.

Chapter 2 : Methodology

2.1. Background

The South Africa ARV roll-out program, initiated in April 2004, has enrolled over 900 000 patients to date(1). The program uses two standardized regimens for all patients accessing care. The first line regimen consists of stavudine (d4T), lamivudine (3Tc), efavirenz (EFV) or nevirapine (NVP) and the second line contains: didanosine (ddI), zidovudine (AZT) and kaletra. Treatment initiation guidelines have changed over time. Initially, patients with a CD4 count <200 cells/mm^3 or WHO clinical stage 4 disease were eligible. A recent update has extended eligibility were patients are eligible for ART if their CD4 count is less than 350 cells/mm^3 irrespective of the clinical stage,CD4 count is less than 350 cells/mm^3 for pregnant women and patients infected with tuberculosis(TB) and HIV, WHO stage 4 irrespective of CD4 count and MDR/XDR-TB patients(7).

This chapter describes the study setting, study design, study population, study sample size, data collection, and description of study variables and data analysis.

2.2. Study Setting

The study was conducted at Aurum Institute for Health Research, an industrial workplace HIV program, in Johannesburg, South Africa using clinic visits data collected between January 2003 and December 2010.The programme started providing ART in November 2002 and has data on approximately 22 000 ART initiators in over 70 ART workplace ART sites. ART delivery sites within this

programme are widely distributed in South Africa, but are concentrated in industrial areas (9).

Routine care included viral load testing and CD4 monitoring every 6 months. HIV resistance testing was not routinely performed, however, resistance results obtained for clinical or research purposes were available for 146 patients.

2.3. Study design, Study population and Sample size

A cohort study was conducted based on the prospective data collection of the HIV workplace programme longitudinal data from Aurum Institute for Health Research. Eligible patients for the study were all HIV infected patients aged 18 years and above who accessed ART care and treatment at the Aurum Institute for Health Research HIV workplace in Johannesburg South Africa between January 2003 and December 2010.The second eligibility criteria was individuals who had undergone HIVDR .A total of about 22,000 patients accessed ART during the study period. The study utilized only the relevant records which met the inclusion criteria during the study period. About 146 patients were assessed. Each individual was followed up a minimum of 1.5 years (Interquartile range [IQR], 1-2).

2.4. Data collection

Data were collected on all registered patients using standardised forms. In addition, central pharmacy distribution records were used to verify medications. Patient data were integrated with laboratory and pharmacy electronic records. The standard forms were also used to capture all clinic measurements.CD4 count and HIV RNA levels were determined before ART initiation, after 6 weeks on ART, and then every

6 months. A program guideline recommended a switch to second-line therapy if two sequential HIV RNA assays were greater than 1000 copies/mL, and if the provider assessed that good adherence was achieved.

The data collected was then transported or faxed to central Aurum Institute for Health Research offices, where they were double-entered into an Microsoft Access database 2010 (Microsoft Corporation, Seattle, USA).The data was first cleaned in Microsoft Access before it was exported to STATA 11.0 (STATA Corporation ,College Station ,Texas, USA) for further cleaning and analysis.

2.5. Definitions of outcomes and explanatory variables

The main outcome variables in the study were HIVDR, virological failure and CD4 cell count slope. These variables were defined as follows;

HIV drug resistance (HIVDR) was defined as high level resistance to one or more agents in an ART regimen using the USA-International Antiretroviral Society (IAS) table of drug resistance mutations. HIVDR was described as a dichotomous variable for the presence or absence of any high level resistance.

Virological failure was defined as viral load of more than 1000 HIV RNA copies per mL for more than six months after initiating first line ART while still taking such treatment.

CD4 slope was defined as the changes of CD4 count cells per mL per year.

The following independent variables were considered: age, gender, history of tuberculosis (TB), baseline WHO clinical stage, baseline CD4 cell count, time

updated CD4 cell count, baseline viral load, time updated viral load and ART experience. These factors were selected based on careful literature review on HIV drug resistance .The Baseline and time updated viral load was categorised into three groups: less than 4 log copies/mL, 4-5 log copies/mL and above 5 log copies/mL. These categories were created because of the observed distribution in the study population. A summary of individual-level variables is presented in **Appendix A1.**

2.6. HIV drug testing

Two types of assays are usually used to assess antiretroviral drug resistance in HIV. Phenotypic and Genotypic assays. Phenotypic drug susceptibility assays determine the inhibitory concentration of the ART agent that reduces HIV-1 replication by 50% (IC50) in tissue culture. Genotypic assays determine the presence of mutations that are known to confer decreased drug susceptibility(29).Since genotypic assays are more convenient and less expensive to carry out, they are used on a larger scale(30).In 2003,every third individual with viremia on the basis of study number was selected. Specimens for genotyping were selected from first detection of viremia and those patients with persistent viremia from subsequent time points for which samples were available. To asses if patients whose samples were genotyped were representative of the larger population, patients with genotype results and patients who developed viremia but did not have genotyping where compared, by means of chi-square test or student t test for categorical and continuous variables respectively.

2.7. Laboratory test

HIV RNA was assayed with the Amplicor HIV-1 Monitor Test (Roche Diagnostics). Sequencing to-date had been performed by extracting viral RNA from stored plasma and amplifying a 1.7Kb fragment spanning the *pol* gene by nested polymerase chain reaction (PCR) assay with the Thermoscript RT-PCR System (Invitrogen). PCR products were sequenced using BigDyeTerminators and an ABI 310 DNA Sequencer (Applied Biosystems)(31). Consensus sequences from all genotyped individuals were aligned and manually edited using the Sequencher program, version 4.5 (GeneCodes). Multiple alignments were performed using Clustal X, version 2.0 (Conway Institute). Phylogenetic analysis of nucleic acid sequences were performed with Mega, version 4.0

2.8. Data validation

Data was double entered and checked in Microsoft Access database 2010. Data cleaning was done by running frequencies and performing logical and range checks.

Internal data validation was undertaken which was the verification of dates of birth, dates of ART initiation, dates of resistance development, dates of CD4 count and viral load measurement. Data validation included review of duplicate records where one record was dropped from analysis if duplicates were found.

2.9. Statistical analysis

Both descriptive and inferential analyses were performed. Descriptive analysis was used to describe the study population using Chi-squared tests, Student t tests and Kruskal Wallis tests. Loss to follow-up, stopping ART, death and transfer out to other clinics were considered as censoring events if an outcome of interest did not occur before the censoring event. The main outcomes of interest were HIVDR, virological failure and CD4 cell count slope.

Since there were three outcomes, three analyses methods were considered: Logistic regression models, Cox proportional hazard models and Linear mixed-effects regression models.

In Logistic regression models, HIVDR was the main outcome variable and was categorised as presence or absence of HIVDR. The other outcomes of interest were the presence or absence of M184V and K103N resistant mutates. The Logistic regression models were used to assess factors associated with the development of HIVDR, M184V and K103N resistance.

Cox proportional hazard models were used to investigate predictors of virological failure in patients who had HIVDR virus. Cox proportional hazard models were appropriate because when applied to censored data and time to event, they account for problems such as attrition, delayed entry and temporal biases. Patients were censored on 31st December 2010. The entry point into the study was when the patient started ART treatment and the exit point when the patient transferred out, died or stopped ART treatment. In univariable analyses, Cox proportional hazard models were used to determine factors associated with the virological failure and

each covariate of interest. The proportional hazard assumption was assessed using Schoenfeld residual test. The observations were considered independent and that there was independent censoring. Multivariable Cox proportional hazard models were used to assess the association between virological failure and independent variables of interest.

To assess factors associated with longitudinal CD4 cell changes, Linear mixed-effects regression were used and CD4 change was modelled as the dependent variable with variation with repeated measures for a patient modelled as a random effect. All estimates were reported with 95% confidence intervals (CI). In all the models used in the analysis, the log-likelihood ratio tests were used to assess contribution of covariates to the models and linear trend for categorical covariates. Missing data were excluded in the assessment of the contribution of each covariate to the models. Baseline characteristics of individuals with or without missing data were compared and the results were comparable. Covariates were entered into a multivariable model if they had p–value of less than 0.20 in univariable analysis, except age and gender which were included *a priori* in the multivariable analysis regardless of statistical significance. The final multivariable model was determined using a backward stepwise regression method and only included covariates with a two-sided p-value of equal to or less than 0.05. Interaction terms were tested in all the models with an interaction terms to a models without interactions using likelihood ratio tests. All interaction terms were not significant so were not included in the final models. For Cox proportional hazard models, evaluation of the fit of the model was done by using the Cox-Snell residuals. Unadjusted hazard ratios (HR) and adjusted HR were obtained with their 95% CI in all the models.

2.10. Ethical considerations

The data collected for this study did not include any personal identifiers and all study participants were identified by unique identifier. Thus no personal identifiers were available in the dataset and the data was analysed anonymously. All patients included in the study signed consent forms. The study was approved by the Research Ethics Committee of the University of Witwatersrand (Human) **Appendix B1**.

Chapter 3 : Results

3.1. Introduction

This chapter presents results from the analysis of a dataset for HIV infected patients aged 18 years and above who were enrolled in the ART programme at Aurum Institute for Health Research between January 2003 and December 2010.The analyses are in three parts; the first part involves describing the cohort, assessment of the factors associated with HIVDR using Logistic regression techniques. Patterns of HIVDR are also presented. The second part examines the risk factors associated with virological failure using Cox proportional-hazard regression models. The third part assesses the risk factors associated with the changes in CD4 cell count using Linear mixed effects regression models, specifically focusing on whether the presence of HIVDR affects CD4 count slope. The chapter begins by a brief description of the baseline characteristics of the study participants.

3.2. Baseline characteristics of the patients enrolled in the study

The baseline characteristics of the study population are shown in **Table 3.1**.Of the 22,208 patients enrolled between January 2003 and December 2010, 10,090 (45%) were women. The median age at ART initiation was 37 years (IQR 31-45). Thirteen thousand and eighty four (59%) patients were aged less than 40 years. At ART initiation, 11,201 (50%) were classified as WHO clinical stage 1 or 2; 5,007 (23%) were classified as stage 3; and 6,000 (27%) were classified as stage 4. A total of

5,619 (25%) patients had no baseline CD4 cell count data. Of those with baseline CD4 cell count data, median CD4 cell count at ART initiation was 135 cells /mm^3 (IQR, 62 – 205). There were 3,879 (17%) patients with baseline viral load of less than 4 $\log_{10}$ copies per mL; 12, 357 (56%) patients had baseline viral load of more than 4 $\log_{10}$ copies per mL; 5,972 (27%) patients had no baseline viral load data and 4,324 (20%) patients were previously on TB treatment.

There were 2,615 (12 %) patients who were already on ART before enrolment into the study. About 12,782(58%) patients were on stavudine; 20, 595 (92%) patients were on lamivudine; 14,383(64%) patients were on efavirenz.

From the total cohort, the study focused on patients with HIVDR data only.

3.3. Patterns of HIV drug resistance in the cohort

Among 22,208 patients enrolled, 146 patients had drug resistance tests performed during the study period. Of these, 108 (74%) developed HIVDR, of whom 28 (26%) were females; the median age was 40 years (IQR, 33-47). Of those with HIVDR, the median CD4 cell count at ART initiation was 121 cells/mm^3 (IQR, 61-210). The baseline characteristics of the patients who developed HIVDR are shown in **Table 3.1.** The median duration on ART was 17 months (IQR, 11-33). About 40 (37%) patients were in WHO stage 3 and 22 (21%) patients were in WHO stage 4. Approximately one-third of the patients (35%) had baseline viral load of more than 5 $\log_{10}$ copies per mL.

Table 3.1 Baseline characteristics of all patients and HIV drug resistance patients at initiation of antiretroviral therapy

Characteristic	All patients (N=22,208)[a]	HIVDR Patients (n=108)
Gender		
Male	12,117(54.56)	80(74.07)
Female	10,090(45.43)	28(25.93)
Age, median years (IQR)	37(31-45)	40(33-47)
WHO Stage		
1 or 2	11,201(50.44)	45(42.06)
3	5,007(22.55)	40(37.38)
4	6,000(27.02)	22(20.56)
CD4 at baseline, cells/μL		
<100	6,270(28.23)	41(37.96)
100-250	7,937 (35.74)	38(35.19)
≥250	7,966(35.87)	29(26.85)
Viral load at baseline, log_{10} copies/mL		
<4	3,879(17.47)	22(20.37)
4-5	7,101(31.97)	31(28.70)
>5	5,256(23.67)	38(35.19)
Unknown	5,256(23.70)	16(15.74)
History of TB at ART initiation		
Yes	4,324(19.45)	40(37.04)
No	17,878(80.50)	68(62.96)
Previously on ART treatment		
Yes	2,615(11.78)	16(14.81)
No	15,867(71.45)	80(74.07)
Unknown	3,726(16.78)	12(11.11)

[a] Unless otherwise indicated, data are reported as number (percentage) of patients.

Of the patients with drug resistance tests, 23 samples had no mutations identified (**Table 3.2)**. The most frequent NNRTI mutations patterns found were mutations

leading to resistance to NNRTI agents with 33 % having NNRTI resistance. The distribution of NNRTI mutations was as follows: K103N (28%), V106M (12%), G190A (7%), P225H (7%), K19E (4%) and Y188L (4%).The second most common resistance pattern was resistance to lamivudine conferred by the M184V mutation (30%).

Table 3.2 Patterns of mutations among patients with HIV drug resistance (n=108)

Mutation pattern [a]	**Total (n)**
NNRTI	50
M184V	48
K103N	30
TAM	17
V106M	13
K70R	11
P225H	8
D67N	8
G190A	8
K65R	7
T215Y	6
V108I	5
A98G	5
V75I	4
Y188L	4
K101E	4
T215F	3
Y115F	3
F227L	3
Wild type virus(no mutations identified)	23

[a] Categories are not mutually exclusive

Among patients with exposure to nevirapine (n=23), the most common mutations were K103N (26%), V106M (4%) and Y188L (4%).There was no patient who was exposed to both nevirapine and efavirenz.

Other mutations conferring NRTI resistance were seen less commonly and these are TAMs (16%).Of the patients with TAM-containing viruses (n=17), the most frequent TAM's were D67N (47%), M41L (41%), T215Y (35%) and K219Q (18%). We also identified seven patients with the K65R mutation, leading to resistance to tenofovir, abacavir and stavudine.

Some resistance patterns have been well described to be associated with specific ART regimens. For example, K65R mutation is most commonly associated with tenofovir exposure while TAMs are most commonly associated with AZT exposure. In this study, patients who were on Zidovudine (69%) had more TAM's than those patients on Stavudine (25%) and Tenofovir (TDF) (6%).

3.4. Risk factors associated with HIV drug resistance

Three types of drug resistant strains were assessed; any resistance defined as presence or absence of any mutation; M184V resistance defined as presence or absence of M184V mutation; K103N defined as presence or absence of K103N mutation. Univariable and multivariable analyses were fitted with these three types of mutations as outcome variables.

In univariable analysis, only CD4 cell count at detection of HIVDR was associated with the emergence of any HIV drug resistance mutation **(Table 3.3)**; gender, age,

WHO stage at baseline, baseline viral load, history of ART treatment at ART initiation and history of TB at ART initiation were not.

The multivariable analysis demonstrated that CD4 cell count at detection of HIVDR was significantly associated with the development of any HIV drug resistance mutation after adjusting for gender and age. At detection of HIVDR, every 200 cells per mL increase in CD4 cell count was associated with a 63% lower odds of detecting any HIV drug resistance adjusting for age and gender (adjusted OR=0.37, 95% CI 0.15 – 0.94). However, there was no evidence of a significant difference in the odds of detecting HIV drug resistance between those patients in WHO stages 3 at baseline (adjusted OR=0.45, 95% CI 0.13 – 1.64) and WHO stage 4 at baseline (adjusted OR=1.39, 95% CI 0.32 – 6.10) compared to those patients in WHO stage 1 or 2 at baseline after adjusting for age and gender. The patients who were previously on ART were almost twice as likely to develop HIV drug resistance compared to those who had never been on ART before at ART initiation (adjusted OR=2.06, 95% CI 0.41 – 10.40) after further adjusting for age and gender, however the effect size was attenuated and lost statistical significance. Similarly, there was no evidence of a difference in the odds of developing HIV drug resistance between those patients with log viral load between 4 and 5 log10 c/mL at virological failure (adjusted OR=0.80, 95% CI 0.29 – 2.24) and log viral load greater than 5 log10 c/mL (adjusted OR=1.26, 95% CI 0.28 – 5.60) compared to those with log viral load of less than 4 log10 c/mL after adjusting for age and gender.

Patients who were on TB treatment before ART initiation were three times more likely to develop any HIV resistant virus compared to those who were not on TB

treatment before after adjusting for age and gender however there was no evidence of significant difference (adjusted OR=2.48, 95% CI 0.67 – 9.09).

Table 3.3 Factors associated with any HIV drug resistance among patients on antiretroviral therapy at the Aurum Health Institute (n=146)

	Any HIV drug resistance			
	Univariable		Multivariable*	
Characteristic	**OR (95 % CI)**	**p-value**	**OR (95 % CI)**	**p-value**
Gender				
Female	1	0.94	1	0.25
Male	0.97(0.42-2.25)		2.00(0.62 -6.43)	
Age, per 10 year increase	0.90(0.58-1.37)	0.61	0.65(0.36-1.14)	0.13
WHO Stage				
1 or 2	1	0.34	1	0.28
3	1.37(0.62-3.02)		0.45(0.13-1.64)	
4	2.15(0.72-6.41)		1.39(0.32-6.10)	
CD4 cell count, per 200 increase [a]	0.56(0.36-0.87)	0.01	0.37(0.15-0.94)	0.04
Viral load, log copies/mL [b]				
0-4	1	0.90	1	0.85
4-5	0.87(0.39-1.98)		0.80(0.29-2.24)	
>5	0.78(0.24-2.52)		1.26(0.28-5.60)	
ART history at ART initiation				
No	1	0.18	1	0.38
Yes	2.28(0.62-8.30)		2.06(0.41-10.40)	
TB history at ART initiation				
No	1	0.17	1	0.17
Yes	1.75(0.78-3.93)		2.48(0.67- 9.09.)	

[a] CD4 at HIVDR detection; [b] viral load at HIVDR detection; * also adjusted for age and gender;

In univariable analysis, CD4 cell count at HIVDR detection, gender, age, WHO stage at baseline, baseline viral load, previously on ART treatment and history of TB at ART initiation were not associated with the emergence of M184V resistance

Table 3.4 Factors associated with M184V and K103N resistance among patients on antiretroviral therapy at the Aurum Health Institute (n=146)

	M184V mutation		K103N mutation	
	Univariable		Univariable	
Characteristic	**OR (95 % CI)**	**P-value**	**OR (95 % CI)**	**P-value**
Gender				
Female	1	0.36	1	
Male	0.68(0.30 -1.56)		2.65(1.12-6.31)	0.03
Age, per 10 year increase	1.08(0.72-1.60)	0.72	0.88(0.54-1.41)	0.58
WHO Stage				
1 or 2	1	0.29	1	0.14
3	1.61(0.73-3.54)		0.89(0.33-2.40)	
4	1.98(0.77-5.10)		2.5 (0.89-6.99)	
CD4 cell count, per 200 increase [a]	1.09(0.75-1.58)	0.67	0.79(0.47-1.34)	0.35
Viral load, log copies/mL [b]				
0-4	1	0.38	1	0.71
4-5	0.84(0.39-1.80)		1.04(0.43-2.54)	
>5	0.41(0.11-1.57)		0.5(0.11-2.69)	
ART history at ART initiation				
No	1	0.72	1	0.05
Yes	1.19(0.43-3.30)		2.98(1.03-8.60)	
TB history at ART initiation				
No	1	0.40	1	0.28
Yes	1.36(0.66- 2.81)		0.60(0.24-1.54)	

[a] CD4 at HIVDR detection; [b] viral load at HIVDR detection

(Table 3.4). Similarly, in multivariable analysis, gender, age, WHO stage, baseline viral load and CD4 at HIVDR detection were not significantly associated with the development of M184V (results not shown).

In univariable analysis, gender and ART history at initiation were significantly associated with the development of K103N resistance. However age, WHO stage,CD4 cell count at HIVDR detection, baseline viral load and history of TB at ART initiation were not associated with the emergence of K103N resistance **(Table 3.4)**; Males were almost three times more likely to develop K103N resistance compared to females and the results were statistically significant (unadjusted OR=2.65, 95% CI 1.22 – 6.31). Patients who were previously on ART were three times more likely to develop K103N resistance compared to those who did not initiate ART treatment before (unadjusted OR=2.98,95% CI 1.03–8.60).

3.5. Risk factors associated with virological failure

Virological failure was defined as viral load of more than 1000 HIV RNA copies per mL for more than six months after initiation of first line HIV treatment while still on treatment. In this study, the virological failure was assessed for all patients who had any HIVDR virus. About 17% (18/108) of the patients who had any mutation developed virological failure.

In univariable analysis, age and CD4 cell count during follow-up (time updated or current CD4 cell count value) were associated with the hazard of virological failure in patients who developed HIVDR. However, age, WHO stage at baseline, viral load at

baseline, ART history at initiation and TB history at initiation were not associated with virological failure in patients who developed HIVDR (**Table 3.5**).

The multivariable analysis showed significant association between age at ART initiation and CD4 cell count during follow-up (time updated) with the hazard of virological failure in those patients who developed HIVDR. However, gender, WHO stage at baseline, viral load at baseline, ART history at initiation and TB history at initiation were not associated with virological failure in patients who developed HIVDR. Every 10 year increase in age was associated with a 29% lower hazard of developing virological failure in patients who developed HIVDR (adjusted HR 0.71, 95% CI 0.52 – 0.97). A 200 CD4 cell count increase was associated with a 46% reduced hazard of virological failure in patients who developed HIVDR (adjusted HR= 0.54 95% CI 0.36 – 0.81).

The hazard of developing virological failure was 8 % higher in patients who were in WHO stage 3 compared to patients who were in WHO stages 1 or 2 (adjusted HR= 1.08 95% CI 0.51 – 2.23) and 4 % higher in patients who were in WHO stage 4 compared to patients in WHO stage 1 or 2 (adjusted HR= 1.04 95% CI 0.53 – 2.02). A linear increasing trend in virological failure was observed with increasing WHO stage (χ^2 = 28.04, p<0.01). There was a consistent linear trend towards increased HIVDR with WHO clinical stages 3 and 4, but that the confidence interval was wide and included the potential for decreased association with resistance. The hazards of developing virological failure was 7% lower in comparing patients with baseline viral load of more than 5 log copies per mL to patients with viral load of less than 4 log

Table 3.5 Factors associated with virological failure among patients with HIV drug resistance at the Aurum Health Institute (n=108)

Characteristic	Virological failure			
	Univariable		Multivariable	
	HR (95 % CI)	P-value	HR (95 % CI)	P-value
Gender				
Female	1	0.05	1	0.23
Male	1.58(0.99-2.53)		1.44(0.79- 2.63)	
Age, per 10 year increase	0.91(0.72-1.12)	0.33	0.71(0.52-0 .97)	0.03
WHO Stage				
1 or 2	1	0.32	1	0.99
3	0.92(0.59-1.42)		1.08(0.51-2.23)	
4	0.75(0.44-1.26)		1.04(0.53-2.02)	
CD4 cell count, per 200 increase [a]	0.61(0.47-0.84)	<0.01	0.54(0.36-0.81)	<0.01
Viral load, log copies/mL [b]				
0-4	1	0.63	1	0.74
4-5	1.33(0.75-2.36)		1.18(0.56- 2.49)	
>5	1.19(0.68-2.05)		0.93(0.48-1.83)	
ART history at ART initiation				
No	1	0.40	1	0.65
Yes	0.79(0.46-1.37)		1.17(0.59-2.32)	
TB history at ART initiation				
No	1	0.30	1	0.60
Yes	0.80(0.53-1.22)		0.84(0.43-1.63)	

[a] CD4 at HIVDR detection; [b] viral load at HIVDR detection.

copies per mL (adjusted HR= 0.93 95% CI 0.48– 1.83) and the hazards of developing virological failure was 18% higher in patients with baseline viral load of between 4 and 5 log copies per mL compared to patients with baseline viral loads of less than 4 log copies per mL(adjusted HR= 1.18 95% CI 0.56 – 2.49).

3.6. Factors associated with CD4 cell count changes

Since understanding the CD4-cell count slope in the setting of virological failure was a major objective of this project, we focused on the 146 patients who had genotypic tests done. These patients contributed about 557 CD4 cell count measurements soon after virological failure and before initiation of an alternative regimen. For this analysis, entry into observation (baseline) was defined as the time when the patients had developed virological failure.

Slope for patients with any resistance was compared to slope of patients without any resistance among patients presenting with treatment failure. Similarly, slopes for patients with NNRTI, M184V, TAM and K103N resistance were compared to slopes for patients with no NNRTI, M184V, TAM and K103N resistance. There was significant association between changes in CD4 cell count and any resistance **(Table 3.6)**. The CD4 cell count slope increased by about 10 cells per mL per year for patients without any resistance (average annual change 9.89 cells per mL, 95% CI -6.90 -26.69) and decreased by 10 cells per mL per year for the patients with any resistance(average annual change -9.62 cells per mL,95 % CI -19.41-0.17). Similarly, there was marginally significant association between the changes in CD4 cell count and TAM resistance (p=0.06). Among patients with virological failure, the CD4 cell count decreased by 4 cells per mL per year for those who had no TAM resistance (average annual change -3.98 cells per mL 95% CI -12.89 -4.93) and a decrease of about 10 cells per mL for those who had TAM resistance (average annual change -9.10 cells per mL 95% CI -34.48-16.28).

But there was no association between changes in CD4 cell count and NNRTI resistance. Among patients with virological failure, the CD4 cell count slope decreased by 2 cells per mL per year for the patients without NNRTI resistance (average annual change -1.67 cells per mL, 95% CI -11.96 -8.62) and by 11 cells per mL per year for the patients with NNRTI resistance (average annual change -10.94 cells per mL, 95% CI -25.02-3.13), however the results were not statistically significant. Similarly, there was no association between changes in CD4 cell count and M184V resistance. Among patients with virological failure, the CD4 cell count declined by 1 cell per mL per year for the patients without M184V resistance (average annual change -0.59 cells per mL, 95 % CI -11.93 -10.74) and declined by 10 cells per mL per year for the patients with M184V resistance (average annual change -10.45 cells per mL, 95% CI -23.16-2.27, however the results were not statistically significant. In addition, there was no association between changes in CD4 cell count and K103N resistance (p=0.50). Among patients with virological failure, the CD4 cell count slope decreased by 3 cells per mL per year for the patients without K103N resistance (average annual change -3.20 cells per mL, 95% CI -12.37 -5.96) and by 17 cells per mL per year for the patients with K103N resistance (average annual change -17.27 cells per mL, 95% CI -42.21-7.67).

Table 3.6 CD4 cell slopes of patients who developed HIV drug resistance at the Aurum HIV workplace (n=108)

	Any resistance		NNRTI resistance		M184V resistance		TAM resistance		K103N resistance	
	Slope (95 % CI)	P	Slope (95 % CI)	P	Slope (95 % CI)	P	Slope (95 % CI)	P	Slope (95 % CI)	P
[a] No Resistance	9.89(-6.90-26.69)	0.05	-1.67(-11.96-8.62)	0.15	-0.59(-11.93-10.74)	0.21	-3.98(-12.89- 4.93)	0.06	-3.20(-12.36-5.96)	0.50
Listed Resistance	-9.62(-19.41-0.17)		-10.94(-25.02-3.13)		-10.45(-23.16-2.27)		-9.10(-34.48-16.28)		-17.27(-42.21-7.67)	

[a] no resistance refers to; no any resistance, no NNRTI, no M184V or no TAM depending on the column. P, p-value

Chapter 4 : Discussions

Making use of a large HIV programme data set, we evaluated HIVDR and changes in CD4 cell counts among patients accessing ART at Aurum in Johannesburg South Africa between January 2003 and December 2010. Specifically, the study assessed HIVDR patterns and factors associated with HIVDR; the association between virological failure and HIVDR resistance and the association between changes in CD4 cell count with HIVDR.

This chapter presents discussions of the findings.

4.1. Patterns of HIV drug resistance

This study documented the prevalence and pattern of HIVDR mutations in a cohort of HIV infected patients who were on first line ART regimen. The results of the study demonstrated the presence of at least one drug resistant HIV mutant from plasma samples obtained from 74% of patients at the time of virological failure during ART treatment. This result is consistent with results from other studies conducted in other African settings. A similar prevalence of ART drug resistance was reported among samples from patients who were on ART in South Africa, Malawi, Uganda and Chad (15, 17, 19, 32-34). The high prevalence of drug resistance (74%) is unsurprising as the NNRTI component of first-line regimens is highly susceptible to rapid development of drug resistance in the setting of abrupt discontinuation of ART or multiple days of missed therapy. However, we found a lower prevalence of mutations leading to resistance to lamivudine and zidovudine than several of the studies. This may be a result of earlier identification of treatment failure in South African HIV

programmes in which HIV RNA assays are routinely done unlike other resource poor settings in which treatment failure is monitored via CD4 count.

The results from the study showed that mutations conferring high level resistance to NNRTI agents were present in viruses from one third of the patients tested for resistance, with K103N and V106M as the most common NNRTI mutations. Mutations conferring high level resistance to NRTI agents were also observed. The second most common resistance pattern found in the study was resistance to lamivudine conferred by the M184V mutation in 30% of patients tested. Other mutations conferring NRTI resistance were seen less commonly and these were TAM's (16%).The drug resistance mutation patterns identified in this study were similar to those reported by other studies (15, 16, 35, 36). However, these results do not support the findings of the previous study conducted in South Africa in 2008 in which a cohort of 313 ART initiated individuals receiving NNRTI and two NRTIs as first line HAART was followed and TAMs did not emerge at all in the patients (37).

4.2. Risk factors associated with HIV drug resistance

Our study findings suggest that CD4 cell count at the time of the detection HIVDR resistance was associated with the development of drug resistance. The odds of developing any drug resistance increased with a decrease in CD4 cell count. Similarly, after adjusting for viral load at the time of HIVDR detection, age at baseline and gender, higher CD4 count at the time of the HIVDR detection was significantly associated with lower odds of HIVDR resistant virus development. This finding agrees with findings of previous studies (25, 27, 38). One possible determinant of the such positive association could be explained by the impaired fitness of resistant virus

(39, 40), leading to reduced pathogenicity and preservation of CD4 cell counts after infection(25). However, our study support those of previous studies suggesting that CD4 cell count was not associated with the presence of either M184V or K103N resistance (19, 41).This result is however is surprising and can be attributed to small sample size.

The data also demonstrate the association between history of ART and the presence of K103N mutation. Those patients who were on ART before the study enrolment were twice more likely to develop K103N mutation compared to those who had never been on ART. This is likely a result of having developed resistance during the prior therapy or after discontinuing the prior therapy that compromises the re-started “first-line” therapy.

The study also showed that gender was associated with the presence of K103N mutation. Males were three times more likely to develop K103N mutation compared to females. The clinical significance of this finding is not known. However, there is a report which demonstrated that the K103N NVP resistance mutation could be detected in women receiving NVP (42). In this present study, we can only speculate that the high risk of developing K103N resistance observed in males could be due to frequent use of nevirapine and efavirenz in this group compared to females.

The study demonstrated that viral load at the time of resistance testing was not a significant predictor of the presence of the HIVDR. This implies that there was no significant association between patients who had low viral load and HIVDR. This finding supports the findings of a cohort study of 239 patients by Booth *et al* (25). However, these results do not support findings of another observational cohort study by Harrigan *et al* in which viral load was found to be significantly associated with

HIVDR(24). Our study and the study by Booth et *al* only included a small sample size compared to the study by Harrigan *et al*. Secondly, certain differences in specific criteria used to define viral load could also contribute to the differences observed between the present study and that of Harrigan *et al*. For example, in this study we used viral load at the time of detection of HIVDR while Harrigan *et al* used baseline viral load.

4.3. Risk factors associated with virological failure

The results of this study demonstrated that time updated CD4 cell count was the significant risk factor of virological failure in patients who developed HIVDR; but not the baseline CD4 cell count. The hazard of developing virological failure in those patients with HIVDR increased with the decline in CD4 cell count. This may be because patients with low CD4 cell counts may have had a longer gap between HIV RNA testing and thus a longer duration of failure (43). This finding supports the findings of previous study conducted in South Africa which also found significant association between CD4 cell count and virological failure(44).Related to this, the study also showed that gender was significantly associated with the development of virological failure in patients who developed HIVDR. Males who had HIVDR were more likely to develop virological failure compared to females who had HIVDR. Similarly, the study further revealed that prior ART at the time of initiating ART in the Aurum programme was not significantly associated with virological failure in patients who developed HIVDR. This finding however does not support the finding of a retrospective cohort study of 362 HIV-infected patients by Sungkanuparph *et al* (45).The difference in the criteria used to define virological failure could explain the

difference between the present study and that of Sungkanuparph *et al.* For instance, in this study virological failure was defined as viral load of more than 1000 HIV RNA copies per mL for more than six months after ART initiating treatment while in the other study virological failure was defined as two consecutive viral load measurements >1000 copies/mL.

4.4. Factors associated with changes in CD4 cell count

The findings of this study suggest that there was an association between the changes in CD4 cell count and the presence of any resistance in those patients who developed virological failure. Those patients who had any resistance had great decrease in CD4 count per year when compared to patients who did not have resistance. Similarly, patients with TAM resistance had more rapid decline in CD4 cell count compared to patients who did not have TAM resistance. It is however unclear why patients with any resistance and TAM resistance had significantly faster CD4 cell decline compared to those who did not have either any resistance or TAM resistance.

In addition, we anticipated that individuals with resistant mutations would have a slower CD4 cell count decline because the presence of mutations may suggest either (1) better ART adherence and/or (2) reduce viral fitness and pathogenicity (46). However, what we found was that individuals with resistant mutations had, on average, a more rapid CD4 cell count decline. We are unsure of how to explain this. Further data on opportunistic infections may be helpful if those individuals had an acute opportunistic infection or tuberculosis disease leading to CD4 cell suppression. Further probing of the data available in this programme dataset may provide a better

understanding. Use of additional data to create a larger dataset may also improve our ability to understand CD4 cell count trajectories during failure among individuals who are still receiving ART.

If this finding holds up under further scrutiny, it has important implications for programmes that do not have access to routine HIV RNA monitoring and use CD4 count criteria for ART failure. The implications of our findings are that development of HIVDR leads to a more rapid decline in CD4 count, while in the absence of HIVDR the CD4 count is more likely to remain stable. As a result, nearly all patients diagnosed with ART failure by CD4 criteria could be expected to have HIVDR. While others who have viremia and virological failure without resistance may be slower to reach CD4 count failure criteria. This may be an explanation for the high proportion of patients with resistance in some studies from Africa (15).

4.5. Limitations of the study

The first limitation of the study was that the study used secondary data and therefore other potential factors associated with drug resistance and virological failure identified in other studies such as treatment adherence and BMI were missing. This limited the ability of the study to assess accurately the factors associated with HIVDR and virological failure.

The second limitation of the study included missing data on very important variables and the relatively modest sample size. This, it is believed, affected the power of the study hence further analysis using larger number of patients is still required.

The third limitation reflected on the nature of the cohort at the time of virological HIV failure. It should be taken into consideration that the cohort included in the study was treatment failing patients who received genotyping and might not be fully representative of all South African patients failing treatment.

A forth limitation is with the analysis of CD4 slope among individuals with and without HIVDR. For most patients we only had a resistance test from a single time point to classify resistance status. During failure, more resistance is likely to develop, thus we likely miss-classified as having no resistance although they had developed resistance during the CD4 observation period.

Finally, because it is an observational programmatic cohort there is considerable heterogeneity in patients and patient care. While advantageous in some ways, including generalizability, the heterogeneity may require a larger sample size to accurately answer the questions we set out to ask. We had a relatively small number of patients with resistance results (146) which limited our power in assessing for associations between HIVDR mutations and clinical and patient characteristics.

4.6. Strengths of the study

To our knowledge, this is one of the few studies to specifically address the relationship between HIVDR and CD4 cell count changes in patients with virological failure using longitudinal patient level data. Therefore, these findings might better reflect the reality in sub-Saharan HV programme thus may be applicable to other similar settings.

Chapter 5 : Conclusions and Recommendations

5.1. Conclusions

The study found that there was high prevalence of HIVDR in the study cohort at the time HIV virological failure. The study also found that the most common mutations in the cohort were NNRTI, M184V and K103N mutations.

Higher CD4 count at detection of HIVDR was found to be significantly associated with lower risk of emergence of any drug resistant virus. Viral load at the time of detection of HIVDR, gender, age at baseline, WHO stage at baseline, ART history at the time of ART initiation and TB history at the time of ART initiation were not associated with the presence of any HIVDR virus. The development of M184V mutation was not associated with gender, age at baseline, WHO at baseline, ART history at the time of ART initiation and TB history at the time of ART initiation. Male gender and ART history at initiation were the main risk factors for the presence of K103N. However, age at baseline, HIV/AIDS WHO staging at baseline, CD4 cell count at detection of HIVDR, viral load at the time of detection of HIVDR, and TB history at the time of ART initiation were not associated with the development of K103N mutation.

Time updated CD4 count and male gender were found to be significantly associated with the development of virological failure in those patients who developed HIVDR. However, age, time updated viral, baseline WHO stage, ART history at initiation and TB history at initiation were not significantly associated with the development of virological failure in patients who developed HIVDR.

The study confirmed that there was indication that patients with virological failure had significantly greater CD4 count declines if any mutation and TAM mutation were present.

However, the study did not identify significant differences in CD4 cell count changes for those patients who had virological failure according to NNRTI mutation, M184V mutation and K103N mutation.

5.2. Recommendations and Policy implications

Given the necessity for lifelong ART for those people infected with HIV and the high replication and mutation rates, the emergence of HIVDR is inevitable in the setting of poor adherence, especially to NNRTI agents. In an effort to minimise the risk of the emerging drug resistance there is, therefore, a need to better understand the factors associated with the development of HIVDR virus. These findings point to a great need for multifaceted approach to target interventions that aim to increase patients CD4 cell count since it is a significant predictor of HIVDR and virological failure. Secondly, the high prevalence of the HIVDR especially in patients who had previously received ART suggests that such patients should be either screened, possibly with HIVDR testing, prior to re-initiation of a first-line regimen.Thirdly, there should be national policy and strategy for monitoring and assessment for drug resistance. Fourthly, more studies are warranted to investigate the factors associated with HIVDR prevention. Lastly, efforts should be directed towards providing healthcare infrastructures that will maximize the effectiveness of treatment and minimize the risk of drug resistance.

Appendices

Appendix A1 A summary of individual-level variables

Variable	Definition	Time of data collection	Variable type	Variable coding
Age	Continuous variable modelled as per 10 year increase at every time on ART (= age/10)	Baseline	Independent	NA
Gender	Categorised as male and female	Baseline	independent	Dummy variables: male or female
WHO clinical stage	WHO clinical stage was categorised as 1, 2, 3 and 4 based on WHO clinical stage criteria	Baseline	Independent	Dummy variables : WHO clinical stage 1 or 2; WHO clinical stage 3; WHO clinical stage 4;
CD4 count at baseline	Continuous variable for baseline CD4 count modelled as 200 cell increase at every time on ART(= CD4 value/200)	Baseline	Independent	NA
Time updated CD4 count	Continuous variable for follow–up CD4 count modelled as 200 cell increase at every time on ART (= CD4 value/200)	Every 6 months visit	Independent	N/A
Viral load at baseline	Log transformed viral load categorised into three groups	Baseline	Independent	Dummy variables for: Less than 4 copies/mL; 4–5 copies/mL; more than 5 copies/mL

Time updated Viral load	Viral load at follow up categorised into 3 groups	Follow up	Independent	Dummy variables for: Less than 4 copies/mL;4–5 copies/mL; more than 5 copies/mL
History of TB	Had TB previously before ART initiation	Baseline	Independent	0 = no;1 = yes
Previously on ART treatment	Started ART before ART registration at Aurum Institute	Baseline	Independent	0 = no;1 = yes
HIV drug resistance	episode of ART drug failure while on ART treatment	every follow-up visit	dependent	0 = no;1 = yes
Virological failure	Single episode of virological failure while on ART treatment	every follow-up visit	dependent	0 = no; 1 = yes
CD4 slope	Change in CD4 cell count after ART initiation	Every 6 months	dependent	NA

Appendix B1 University of the Witwatersrand Human Research Ethics Clearance Certificate for the study

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Mr Dickman Gareta

CLEARANCE CERTIFICATE	M111133
PROJECT	Risk Factors associated with Antiretroviral in HIV Patients on Antiretroviral Therapy in South Africa
INVESTIGATORS	Mr Dickman Gareta.
DEPARTMENT	School of Public Health
DATE CONSIDERED	28/10/2011
M1111330DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 28/10/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Edmoew Marinda

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

References

1. UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2010. 2010.

2. WHO, UNICEF, UNAIDS. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector. Progress report 2010. Geneva, World Health Organization, 2010.

3. Tobin NH, Frenkel LM. Human immunodeficiency virus drug susceptibility and resistance testing. The Pediatric infectious disease journal. 2002;21(7):681-3. Epub 2002/09/19.

4. WHO. HIV drug resistance. World Health Organisation; 2012 [08 April 2012]; Available from: http://www.who.int/hiv/topics/drugresistance/en/index.html.

5. National Department of Health D. Operational plan for comprehensive HIV and AIDS care,managemnt and treatment for South Africa. 2003.

6. Johnson LF. Access to antiretroviral treatment in South Africa, 2004 - 2011. Southern African Journal of HIV Medicine. 2012;13(1).

7. National Department of Health D. Clinical guidelines for the management of HIV & AIDS in Adults and adolescents 2010.

8. WHO. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. 2010 revision. 2010.

9. Charalambous S, Grant AD, Day JH, Pemba L, Chaisson RE, Kruger P, et al. Establishing a workplace antiretroviral therapy programme in South Africa. AIDS care. 2007;19(1):34-41. Epub 2006/11/30.

10. Wallis C, Mellors,JW,Venter,WDF,Sanne, I,Stevens, W. Varied Patterns of HIV-1 Drug Resistance on Failing First-Line Antiretroviral Therapy in South Africa. Journal of Acquired Immune Deficiency Syndromes. 2010;53(4):480-4.

11. WHO. HIV Drug resistance fact sheet. 2012 [cited 2012 17 April 2012]; Available from: http://www.who.int/hiv/facts/drug_resistance/en/index.html.

12. van de Vijver D, Wensing A, Boucher C. The Epidemiology of Transmission of Drug Resistant HIV-1. 2008.

13. Tamalet C, Fantini J, Tourres C, Yahi N. Resistance of HIV-1 to multiple antiretroviral drugs in France:A 6 year survey (1997-2002) based on an analysis of over 7000 genotypes.AIDS AIDS. 2003;17(16):2383-8.

14. Richman DD, Morton SC, Wrin T, Hellmann N, Berry S, Shapiro MF, et al. The prevalence of antiretroviral drug resistance in the United States. AIDS. 2004;18(10):1393-401. Epub 2004/06/17.

15. Hosseinipour MC, van Oosterhout JJ, Weigel R, Phiri S, Kamwendo D, Parkin N, et al. The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy. AIDS. 2009;23(9):1127-34. Epub 2009/05/07.

16. Marconi VC, Sunpath H, Lu Z, Gordon M, Koranteng-Apeagyei K, Hampton J, et al. Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa. Clin Infect Dis. 2008;46(10):1589-97. Epub 2008/04/19.

17. Weidle PJ, Malamba S, Mwebaze R, Sozi C, Rukundo G, Downing R, et al. Assessment of a pilot antiretroviral drug therapy programme in Uganda: patients' response, survival, and drug resistance. Lancet. 2002;360(9326):34-40. Epub 2002/07/13.

18. Delaugerre C, Warszawski J, Chaix M, Veber F, Macassa E, Buseyne F, et al. Prevalence and Risk Factors Associated with Antiretroviral Resistance in HIV-1 infected Children. JMedVirol. 2007.

19. Hoffmann CJ, Charalambous S, Sim J, Ledwaba J, Schwikkard G, Chaisson RE, et al. Viremia, resuppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line antiretroviral therapy in South Africa. Clin Infect Dis. 2009;49(12):1928-35. Epub 2009/11/17.

20. Harrigan PR, Wynhoven B, Brumme ZL, Brumme CJ, Sattha B, Major JC, et al. HIV-1 drug resistance: degree of underestimation by a cross-sectional versus a longitudinal testing approach. J Infect Dis. 2005;191(8):1325-30. Epub 2005/03/19.

21. Shepherd J, Etiebet M, Griffifth D, Mondal P, Charurat M, Elegba O, et al. High levels of class-wide NRTI resistance among HIV-positive patients failing first-line anti-retroviral regimens in Nigeria. Abstract no. WEPE0031. AIDS 2008 - XVII International AIDS Conference; Mexico2008.

22. Sungkanuparph S, Manosuth W, Kiertiburanakul S, Piyavong B, Chumpathat N, Chantratita W. Options for a Second-Line Antiretroviral Regimen for HIV Type 1–Infected Patients Whose Initial Regimen of a Fixed-Dose Combination of Stavudine,Lamivudine, and Nevirapine Fails. Clin Infect Dis. 2006;44(3):447-52.

23. Hamers RL, Siwale M, Wallis CL, Labib M, van Hasselt R, Stevens WS, et al. HIV-1 drug resistance mutations are present in six percent of persons initiating antiretroviral therapy in Lusaka, Zambia. J Acquir Immune Defic Syndr. 2010;55(1):95-101. Epub 2010/06/30.

24. Harrigan PR, Hogg RS, Dong WW, Yip B, Wynhoven B, Woodward J, et al. Predictors of HIV drug-resistance mutations in a large antiretroviral-naive cohort initiating triple antiretroviral therapy. J Infect Dis. 2005;191(3):339-47. Epub 2005/01/06.

25. Booth CL, Garcia-Diaz AM, Youle MS, Johnson MA, Phillips A, Geretti AM. Prevalence and predictors of antiretroviral drug resistance in newly diagnosed HIV-1 infection. J Antimicrob Chemother. 2007;59(3):517-24. Epub 2007/01/11.

26. Biagetti C, Bon I, Vitone F, Schiavone P, Borderi M, Pavoni M, et al. Prevalence of antiretroviral drug resistance in untreated persons newly diagnosed with HIV-1 infection. New Microbiol. 2009;32(2):129-34. Epub 2009/07/08.

27. Di Giambenedetto S, Zazzi M, Corsi P, Gonnelli A, Di Pietro M, Giacometti A, et al. Evolution and predictors of HIV type-1 drug resistance in patients failing combination antiretroviral therapy in Italy. Antivir Ther. 2009;14(3):359-69. Epub 2009/05/29.

28. von Wyl V, Yerly S, Boni J, al. e. Emergence of HIV-1 drug resistance in previously untreated patients initiating combination antiretroviral treatment: a comparison of different regimen types. . Arch Intern Med. 2007;167:1782-90.

29. Hanna G, D'Aquila,RT. Clinical Use of Genotypic and Phenotypic Drug Resistance Testing to Monitor Antiretroviral Chemotherapy. Clin Infect Dis. 2001;32(5):774-82.

30. Deshpande A, Jauvin,V,Magnin,N, Pinson, P,Faure,M,Masquelier,B,Aurillac-Lavignolle,V,Fleury,HJ. Resistance Mutations in Subtype C HIV Type 1 Isolates from Indian Patients of Mumbai Receiving NRTIs Plus NNRTIs and Experiencing a Treatment Failure: Resistance to AR. AIDS Research and Human Retroviruses. 2007;23(2):335-40.

31. Pillay V, Ledwaba J, Hunt G. Antiretroviral drug resistance surveillance among drug-naive HIV-1–infected individuals in Gauteng Province, South Africa in 2002 and 2004. . antivir Ther. 2008;13(Suppl 2)::101–7.

32. Koyalta D, Charpentier C, Beassamda J, Rey E, Si-Mohamed A, Djemadji-Oudjeil N, et al. High frequency of antiretroviral drug resistance among HIV-infected adults receiving first-line highly active antiretroviral therapy in N'Djamena, Chad. Clin Infect Dis. 2009;49(1):155-9. Epub 2009/06/02.

33. Singh A, Sunpath H, Green TN, Padayachi N, Hiramen K, Lie Y, et al. Drug resistance and viral tropism in HIV-1 subtype C-infected patients in KwaZulu-Natal, South Africa: implications for future treatment options. J Acquir Immune Defic Syndr. 2011;58(3):233-40. Epub 2011/06/29.

34. Hamers RL, Sigaloff KC, Wensing AM, Wallis CL, Kityo C, Siwale M, et al. Patterns of HIV-1 Drug Resistance After First-Line Antiretroviral Therapy (ART) Failure in 6 Sub-Saharan African Countries: Implications for Second-Line ART Strategies. Clin Infect Dis. 2012;54(11):1660-9. Epub 2012/04/05.

35. Wallis CL, Mellors JW, Venter WDF, Sanne I, Stevens W. Protease Inhibitor Resistance Is Uncommon in HIV-1 Subtype C Infected Patients on Failing

Second-Line Lopinavir/r-Containing Antiretroviral Therapy in South Africa. AIDS Research and Treatment. 2011;2011:5.

36. Luebbert J, Tweya H, Phiri S, Chaweza T, Mwafilaso J, Hosseinipour MC, et al. Virological Failure and Drug Resistance in Patients on Antiretroviral Therapy After Treatment Interruption in Lilongwe, Malawi. Clin Infect Dis. 2012. Epub 2012/05/11.

37. Barth RE, Wensing AM, Tempelman HA, Moraba R, Schuurman R, Hoepelman AI. Rapid accumulation of nonnucleoside reverse transcriptase inhibitor-associated resistance: evidence of transmitted resistance in rural South Africa. AIDS. 2008;22(16):2210-2. Epub 2008/10/04.

38. El-Khatib Z, Delong AK, Katzenstein D, Ekstrom AM, Ledwaba J, Mohapi L, et al. Drug resistance patterns and virus re-suppression among HIV-1 subtype C infected patients receiving non-nucleoside reverse transcriptase inhibitors in South Africa. Journal of AIDS & clinical research. 2011;2(117). Epub 2011/09/20.

39. Devereux HL, Emery VC, Johnson MA, Loveday C. Replicative fitness in vivo of HIV-1 variants with multiple drug resistance-associated mutations. J Med Virol. 2001;65(2):218-24. Epub 2001/09/06.

40. Nijhuis M, Deeks S, Boucher C. Implications of antiretroviral resistance on viral fitness. Current opinion in infectious diseases. 2001;14(1):23-8. Epub 2002/04/30.

41. Vallejo A, Ruiz-Mateos E, Molina-Pinelo S, Soriano-Sarabia N, de Felipe B, Gutierrez S, et al. Immunovirologic characteristics of human immunodeficiency virus-infected patients consisting mainly of injecting drug users on highly active antiretroviral treatment with prolonged virologic failure. Viral immunology. 2006;19(4):759-67. Epub 2007/01/05.

42. Jackson JB, Becker-Pergola G, Guay LA, Musoke P, Mracna M, Fowler MG, et al. Identification of the K103N resistance mutation in Ugandan women receiving nevirapine to prevent HIV-1 vertical transmission. AIDS. 2000;14(11):F111-5. Epub 2000/09/13.

43. Price DA, Scullard G, Oxenius A, Braganza R, Beddows SA, Kazmi S, et al. Discordant outcomes following failure of antiretroviral therapy are associated with substantial differences in human immunodeficiency virus-specific cellular immunity. Journal of virology. 2003;77(10):6041-9. Epub 2003/04/30.

44. Datay MI, Boulle A, Mant D, Yudkin P. Associations with virologic treatment failure in adults on antiretroviral therapy in South Africa. J Acquir Immune Defic Syndr. 2010;54(5):489-95. Epub 2010/04/17.

45. Sungkanuparph S, Groger RK, Overton ET, Fraser VJ, Powderly WG. Persistent low-level viraemia and virological failure in HIV-1-infected patients

treated with highly active antiretroviral therapy. HIV Med. 2006;7(7):437-41. Epub 2006/08/24.

46. Castagna A, Danise A, Menzo S, Galli L, Gianotti N, Carini E, et al. Lamivudine monotherapy in HIV-1-infected patients harbouring a lamivudine-resistant virus: a randomized pilot study (E-184V study). AIDS. 2006;20(6):795-803. Epub 2006/03/22.